CTR GRANT # 310

PROGRESS REPORT NO. 3

RICHARD J. BING, M.D.
Huntington Memorial Hospital
100 Congress Street
Pasadena, California 91105

June 30, 1972 - July 1, 1973

THE EFFECT OF NICOTINE ON LIPID SYNTHESIS AND CHOLESTEROL TRANSFER IN HUMAN CORONARY ARTERIES AND HUMAN SAPHENOUS VEINS

The primary purpose of our work, as sponsored by the Council for Tobacco Research, was to investigate the effect of nicotine on lipid metabolism in human coronary arteries. Special emphasis of this work was on human blood vessels, since considerable differences exist in lipid metabolism of arteries in various species. The emphasis on human material introduced some difficulties; although it was possible to obtain sufficient atherosclerotic human coronary arteries, the supply of normal nonatherosclerotic human coronary arteries was limited. However, it was finally possible to obtain sufficient data to compare diseased and normal human coronary arteries.

We also extended our investigation to the effect of nicotine on the lipid metabolism in perfused coronary saphenous veins. The reason for this was twofold: (1) saphenous veins are more readily available, and we found that the lipid synthesis in the veins differs little from that of coronary arteries; (2) saphenous veins are now frequently employed in the construction of aortocoronary vein grafts,

in order to furnish an increased blood supply to the ischemic heart muscle of the patient with coronary artery disease. Several publications have resulted from these studies, one published in the Proceedings of the Society of Experimental Biology and Medicine, the other (enclosed) submitted to Atherosclerosis.

The first portion of this project was concerned with a study of lipid synthesis and cholesterol transfer into atherosclerotic human coronary arteries. The technique used in these studies consisted of sterile perfusion in a modified Carrel-Lindbergh perfusion apparatus of arteries obtained within 5 hours after death of the patient. Perfusion was carried out for 4 hours with a pressure of 130/100 mmHg and a pulsatile rate of 80. The advantage of the Lindbergh pump is that it is possible to obtain, under sterile conditions, varying degrees of pressure and pulse rate. All vessels were perfused with human plasma, and the gas mixture consisted of 75% nitrogen, 20% oxygen, and 5% carbon dioxide.

Since we were interested in studying lipid synthesis as well as cholesterol transfer, we added 2-^{14}C-sodium acetate and cholesterol-1,2-^{3}H (^{3}H-cholesterol) to the perfusion fluid. If synthesis of lipids occurs, then the acetate will be incorporated into lipids. On the other hand, if cholesterol is being transferred in toto into the vascular wall, then ^{3}H-cholesterol can be traced into the vascular wall. The main difficulty at the onset consisted

of bringing cholesterol into solution. This was finally possible by sonication. The cholesterol was added to a very small amount of concentrated lipid extract from human serum, the mixture was then evaporated in vacuo, and after addition of 5ml of human plasma, the mixture was sonicated three times for 1 minute each at intervals of about 1 minute. Using this procedure, we were able to demonstrate that the cholesterol is bound to the alpha- and beta-lipoprotein fractions of the perfusing serum.

Nicotine (6 mg of nicotine dissolved in 5ml of saline solution) was added prior to adjusting the final volume of 250 ml with human plasma. Nicotine was added to one perfusion system and the other perfusion pump served as control. In the control experiments, saline was used in place of nicotine.

Lipid analysis of the artery was carried out by means of extraction by Folch mixture and, after refluxing, separating it on a thin-layer plate of silica gel according to the method of Freeman and West. After separation of the fractions, they were eluted with eluting solvents, and the activity was counted in a tricarb liquid scintillation spectrometer.

Cholesterol was determined by the method of Zak and coworkers in a Kintrac spectrophotometer. The details of the method are described in our publications (see enclosure). The results on atherosclerotic coronary arteries may be summarized as follows: (1) atherosclerotic human coronary arteries cannot synthesize

cholesterol from acetate; (2) only very limited synthesis of cholesterol esters from acetate occurs; (3) free cholesterol is taken up by the artery; (4) nicotine has only a very slight effect on lipid synthesis from acetate, it has no effect on free cholesterol uptake. It may be mentioned at this point that in normal coronary arteries, as well as in saphenous veins perfused at either low venous or higher arterial pressure, nicotine had no influence on either synthesis of lipids or on uptake of cholesterol or cholesterol esters.

The experiments on coronary arteries, therefore, demonstrate that human coronary arteries differ in many respects from animal coronary arteries and that many of the data accumulated in the literature on animals do not apply to human vessels. Of prime importance was the finding that cholesterol is not synthesized in the human artery but is passively transferred into the vascular wall, as first suggested by Virchow.

We subsequently started a series of experiments in which we investigated lipid metabolism and the effect of nicotine on perfused human nonatherosclerotic coronary arteries and saphenous veins. As mentioned above, it was difficult to obtain a sufficiently large series of normal human coronary arteries, but there has been no lack of saphenous veins because of the popularity of coronary artery surgery.

The technique used in these experiments was similar to that described above. Saphenous veins were removed from patients prior to performing aortic coronary saphenous grafts. The human coronary arteries were obtained from individuals within 5 hours after death. The oldest of these individuals was 18 years old, most of them being children who had succumbed to malignant lymphomas. Here again, the effect of nicotine on lipid synthesis and cholesterol transfer was studied in human saphenous veins perfused at a pressure of 45/35 mmHg. Nicotine tartrate was added to the perfusion fluid to make a final concentration of 150 μM. We were interested in investigating whether or not the uptake of cholesterol by saphenous veins differed in vessels perfused at low as compared to high pressure. This question is of some importance since in coronary surgery the venous graft is exposed to arterial pressures.

In principle, the results did not differ greatly from those obtained on atherosclerotic human coronary arteries. To summarize, (1) human coronary arteries, without evidence of atherosclerosis, did not differ from atherosclerotic arteries in their inability to synthesize cholesterol from acetate; (2) arteries with and without atherosclerotic lesions take up cholesterol from the perfusion fluid to an equal degree; (3) human saphenous veins perfused at relatively low pressure (45/35 mmHg) do not differ from atherosclerotic and normal coronary arteries in their ability

to synthesize lipids; (4) the uptake of cholesterol, however, is less than that of coronary arteries or of saphenous veins perfused at arterial pressures (130/100 mmHg); (5) human saphenous veins perfused at arterial pressures (130/100 mmHg) take up cholesterol from the perfusion fluid equal to that taken up by coronary arteries; (6) in saphenous veins perfused at high pressure, synthesis of lipids from acetate equals that in saphenous veins perfused at lower pressure or of nonatherosclerotic coronary arteries; (7) nicotine failed to influence either synthesis of lipids or uptake of cholesterol.

As to be expected, the uptake of cholesterol was significantly greater in coronary arteries as compared to the saphenous veins perfused at low pressure. This suggests that hemodynamic conditions influence the uptake of cholesterol by the vein from the perfusion fluid; this is in line with the idea of filtration or imbibition of cholesterol. It is also in agreement with the findings of others, such as Werthessen, who found that when calves' aortas were perfused at high perfusion pressure, the amount of cholesterol accumulated by the aorta was limited. The position of cholesterol in areas of the aorta exposed to turbulence is well known. It is believed that the increased uptake of cholesterol in these areas may be the direct result of hemodynamic damage and/or interaction of

foreign elements, particularly platelets with endothelium at points of disturbed flow, as suggested by Packham. The role which permeability of the vascular wall plays in the uptake of cholesterol is not clear, and further experiments are planned to investigate this particular problem. It has been found however, that increased influx of Evan's blue in the intima occurs with increased pressure or wall strain and increased shearing stress; it appears that the increased flux of this dye into the vascular wall parallels augmented uptake of free cholesterol. Possibly, it is the circumferential tension which acts as a determinant of transendothelial passage of proteins by widening the endothelial intercellular spaces through which the transfer can occur.

The finding of increased cholesterol uptake by veins exposed to high perfusion pressure may have significance in determining the eventual fate of aortic coronary saphenous grafts. We believe that this is now being recognized by surgeons, who prefer, if at all possible, an arterial over a vein graft. Finally, the experiments indicate that within the limits of our experiments, nicotine has no effect on the synthesis or the uptake of lipids by human coronary arteries.

It may be seen from the bibliography that a total of 9 papers have been supported by the Council for Tobacco Research during the last year.

BIBLIOGRAPHY
Supported by the CTR

1. T. Morita, and R.J. Bing: Lipid metabolism in perfused human coronary arteries, Proc. Soc. Exp. Biol. Med., 140:617, 1972.

2. H. Hashimoto, J.S.M. Sarma, J. Mao, E. Larsen, and R.J. Bing: Lipid metabolism in perfused human nonatherosclerotic coronary arteries and saphenous veins. Submitted to Atherosclerosis.

3. R.J. Bing, and K. Hellberg: Measurement of coronary flow in ischemic heart disease (edited by W. Likoff, B.L. Segal, W. Insull, Jr.), in Atherosclerosis and Coronary Heart Disease, page 146, 1972, Grune & Stratton, Inc.

4. R.J. Bing, K. Hellberg, and O. Pachinger: Recognition of coronary artery disease, New York State Journal of Medicine, 72:1818, 1972.

5. G. Stefan, and R.J. Bing: Echocardiographic findings in experimental myocardial infarction of the posterior left ventricular wall, the American Journal of Cardiology, 30:629, 1972.

6. R.J. Bing, and K. Hellberg: Coronary blood flow in relation to angina pectoris, Circulation 46:1146, 1972.

7. O.M. Pachinger, and R.J. Bing: Coronary blood flow assessment with xenon and rubidium. Submitted to Seminars in Nuclear Medicine.

8. R.J. Bing, and K. Hellberg: Pharmacodynamics of the coronary microcirculation (edited by J.P. Davidson), in New Perspectives in Diagnosis and Management of Myocardial Infarction, Williams & Wilkins Co., in press.

9. R.J. Bing, H. Wayland, A. Rickart, and K. Hellberg: Studies on the coronary microcirculation by direct visualization. To be published in the Russian Journal of Cardiology.